

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
27 May 2004 (27.05.2004)

PCT

(10) International Publication Number  
**WO 2004/043492 A1**

(51) International Patent Classification<sup>7</sup>: A61K 47/48

SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA,  
UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(21) International Application Number:  
PCT/EP2003/012514

(84) Designated States (*regional*): ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(22) International Filing Date:  
10 November 2003 (10.11.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
02025315.9 13 November 2002 (13.11.2002) EP

#### Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*
- *of inventorship (Rule 4.17(iv)) for US only*

(71) Applicant (*for all designated States except US*): BRACCO IMAGING S.P.A. [IT/TT]; Via Egidio Folli, 50, I-20134 Milano (IT).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): DE HAEN, Christoph [IT/TT]; Bracco Imaging S.p.A., Via Egidio Folli, 50, I-20134 Milano (IT). MAISANO, Federico [IT/TT]; Bracco Imaging S.p.A., Via Egidio Folli, 50, I-20134 Milano (IT).

(74) Agent: MACCHETTA, Francesco; Bracco Imaging S.p.A., Intellectual Property Department, Via Egidio Folli, 50, I-20134 Milano (IT).

#### Published:

- *with international search report*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC,

(54) Title: METHOD FOR THE SELECTIVE AND QUANTITATIVE FUNCTIONALIZATION OF IMMUNOGLOBULIN FAB FRAGMENTS, CONJUGATE COMPOUNDS OBTAINED WITH THE SAME AND COMPOSITIONS THEREOF

(57) Abstract: The invention provides chemical conjugates between an immunoglobulin Fab fragment and molecular entities imparting diagnostic or therapeutic utility, whereby the only sites of conjugation on the Fab fragment are one or both of the sulfhydryl groups deriving from the selective and quantitative reduction of the inter-chain disulfide bond of said Fab fragment and whereby said molecular entities imparting diagnostic or therapeutic utility have at least one free sulfhydryl-reactive group, characterized in that the conjugation stoichiometric molar ratio molecular entity to Fab fragment is in the range from 0.95 to 1.05 or in the range from 1.95 to 2.05. The invention also provides a process for preparing said conjugates and pharmaceutical compositions thereof.



WO 2004/043492 A1

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ARTEAGA DE MURPHY C ET AL: "PHOSPHINE REDUCED IGG: A NEW METHOD FOR 99MTC LABELING IMMUNOGLOBULINS" JOURNAL OF RADIOANALYTICAL AND NUCLEAR CHEMISTRY, ARTICLES, ELSEVIER SEQUOIA S.A., LAUSANNE, CH, vol. 220, no. 1, 1997, pages 41-45, XP000199389 the whole document  --- -/--	1-22

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- \*&\* document member of the same patent family

Date of the actual completion of the international search

1 March 2004

Date of mailing of the international search report

22/03/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Vadot, P

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>BURNS J A ET AL: "Selective reduction of disulfides by tris(2-carboxyethyl)phosphine" JOURNAL OF ORGANIC CHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, US, vol. 56, no. 8, 1991, pages 2648-2650, XP002149302 ISSN: 0022-3263 cited in the application Title Introduction part</p>	1-22
X	<p>SINGH RAJEEVA ET AL.: "Labeling of antibodies by in situ modification of thiol groups generated from selenol-catalyzed reduction of native disulfide bonds." ANALYTICAL BIOCHEMISTRY, vol. 304, - 2002 pages 147-156, XP001165655 Page 149, Paragraph "materials and methods", Biotinylation of antibody by selenol/TCEP reduction of native disulfide bonds to thiol groups... Paragraph "results", "discussion" page 148, left-hand column, paragraph 2; figure 1</p>	1-22
Y	<p>ANELLI ET AL: "L-Glutamic acid and L-lysine as useful building blocks for the preparation of bifunctional DTPA-like ligands" BIOCONJUGATE CHEMISTRY, vol. 10, no. 1, 1999, pages 137-140, ABSTRACT, XP002115959 cited in the application XP002148891 the whole document</p>	1-22
X	<p>SEITZ U ET AL: "PREPARATION AND EVALUATION OF THE RHENIUM-188-LABELLED ANTI-NCA ANTIGEN MONOCLONAL ANTIBODY BW 250/183 FOR RADIOIMMUNOTHERAPY OF LEUKAEMIA" EUROPEAN JOURNAL OF NUCLEAR MEDICINE, BERLIN, DE, vol. 26, no. 10, October 1999 (1999-10), pages 1265-1273, XP000952569 ISSN: 0340-6997 cited in the application Paragraph "materials and methods", Radiolabelling of antibody with 188Re. Paragraph Results, reduction of antibody and labelling with 188Re Discussion part</p>	1-22

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 274 119 A (FRAZIER KEVIN A ET AL) 28 December 1993 (1993-12-28) cited in the application figure 2 ---	1-22
Y	WO 91 04056 A (IMMUNOMEDICS INC) 4 April 1991 (1991-04-04) claims ---	1-22
Y	YASUSHI FUJIOKA, ET AL.: "Renal metabolism of 3'-iodohippuryl N-maleoyl -L-Lysine (HML)-conjugated Fab fragments" BIOCONJUGATE CHEMISTRY, vol. 12, 2001, pages 178-185, XP001165761 cited in the application paragraph 'INTRODUCTION!; figure 3B -----	1-22

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5274119	A	28-12-1993	NONE	
WO 9104056	A	04-04-1991	AT 110281 T	15-09-1994
			AU 640698 B2	02-09-1993
			AU 6521490 A	18-04-1991
			CA 1340250 C	15-12-1998
			DE 69011789 D1	29-09-1994
			DE 69011789 T2	15-12-1994
			DK 419203 T3	05-12-1994
			EP 0419203 A1	27-03-1991
			ES 2063286 T3	01-01-1995
			FI 921146 A	17-03-1992
			IE 903360 A1	10-04-1991
			IL 95723 A	31-08-1995
			JP 7023326 B	15-03-1995
			JP 4505455 T	24-09-1992
			KR 9615611 B1	18-11-1996
			NO 920853 A	04-03-1992
			US 5612016 A	18-03-1997
			WO 9104056 A1	04-04-1991
			US 5601825 A	11-02-1997
			US 5328679 A	12-07-1994
			US 5541297 A	30-07-1996
			ZA 9007420 A	31-07-1991